Claims Appendix

92. (New) A method for the accelerated production of transgenic animals comprising:

a) transfecting a first non-human differentiated somatic cell or cell-line with a transgene construct containing a first DNA sequence;

b) selecting a transfected cell or cell-line into which said first DNA sequence has been inserted into the genome of said first non-human differentiated somatic cell or cell-line;

c) performing a first nuclear transfer procedure to generate a first transgenic animal at least heterozygous for said first DNA sequence;

d) performing a biopsy or other cell selection technique to obtain cells to establish a second non-human differentiated somatic cell or cell-line from said first transgenic animal;

e) characterizing said second non-human differentiated somatic cell or cell-line using known molecular biology methods to ensure that the selected said second non-human differentiated somatic cell or cell-line is at least heterozygous for said first DNA sequence; and

f) performing a second nuclear transfer procedure with at least one of said second non-human differentiated somatic cells to produce at least a second transgenic animal at least heterozygous for said first DNA sequence.

93. (New) The method of claim 92, wherein said first transgenic animal is at an embryonic stage of development.

94. (New) The method of claim 92, wherein said first transgenic animal is at a fetal stage of development.

95. (New) The method of claim 92, further comprising developing said first transgenic animal into an adult non-human animal.

96. (New) The method of claim 92, wherein said first transgenic animal is a mammal.

97. (New) The method of claim 92, wherein said first DNA sequence encodes a desired protein;

98. (New) The method of claim 92, wherein the genetic composition of said first transgenic animal is characterized to confirm the presence and expression of the transgene.

99. (New) The method of claim 92, wherein said first nuclear transfer procedure further comprises transferring the nucleus of said transfected cell into a suitable enucleated recipient cell of the same species, thereby obtaining a reconstituted cell.

100. (New) The method of claim 92, wherein said first transgenic animal is biopsied so as to characterize the genome of said first transgenic animal.

101. (New) The method of claim 92, wherein at least one of the cells from said second non-human differentiated somatic cell or cell-line is expanded through cell culture techniques for use in said second round of nuclear transfer so as to produce a multiplicity of animals transgenic for said DNA of interest.

102. (New) The method of claim 96, wherein the source of said differentiated somatic cell or cell-line is an ungulate.

103. (New) The method of either claims 102, wherein said differentiated somatic cell or cell-line is from an ungulate selected from the group consisting of bovine, ovine, porcine, equine, caprine and buffalo.

104. (New) A method of preparing a genetically engineered transgenic mammal, comprising:

(a) inseminating a first female non-human mammal recipient with semen from a transgenic non-human animal of the same species known to have a transgene present and expressed;

(b) obtaining a transgenic non-human embryo from said first female recipient;

(c) obtaining a somatic cell from said embryo;

(d) culturing said differentiated somatic cell in a suitable medium, such that a differentiated somatic cell line is obtained and,

(e) performing a nuclear transfer procedure with said non-human differentiated somatic cells to produce at least one transgenic mammal at least heterozygous for said first DNA sequence;

wherein said first DNA sequence encoding a desired gene is actuated by a tissue specific promoter.

105. (New) The resultant offspring of the methods of claim 104.

106. (New) The method of claim 92, wherein said second non-human differentiated somatic cell or cell-line cells are obtained from an embryonic goat on or after day 10 of embryogenesis.

107. (New) The method of claim 92, wherein said second non-human differentiated somatic cell or cell line preparation is kept in an airtight container.

108. (New) The method of claim 92 wherein said first DNA sequence codes for a biopharmaceutical protein product.

109. (New) The method of claim 108 wherein said first DNA sequence encoding a desired gene is actuated by at least one beta casein promoter.

110. (New) The resultant milk derived from the offspring of the methods of claim 108.

111. (New) The method of claim 92, wherein said second non-human differentiated somatic cell or cell-line is obtained from said first transgenic animal by known tissue dissociation means including enzymatic means and/or mechanical means.

112. (New) The method of claim 92, wherein said second non-human differentiated somatic cell or cell-line is selected from a group of cell types present in said first transgenic animal including:

a) fibroblasts
b) cumulus cells
c) neural cells
d) mammary cells; and
e) myocytes.

113. (New) The resultant offspring of the methods of claim 92.

114. (New) The method of claim 104 wherein said transgene codes for a biopharmaceutical protein product.

115. (New) The method of claim 114 wherein said tissue specific promoter is a beta casein promoter.

116. (New) The resultant milk derived from the offspring of the methods of claim 114.

117. (New) The method of claim 104, wherein said second non-human differentiated somatic cell or cell-line is obtained from said first transgenic animal by known tissue dissociation means including enzymatic means and/or mechanical means.

118. (New) The method of claim 104, wherein said second non-human differentiated somatic cell or cell-line is selected from a group of cell types present in said first transgenic animal including:

a) fibroblasts

b) cumulus cells

c) neural cells

d) mammary cells; and

e) myocytes.

119. (New) The method of claim 92, wherein said transgene construct comprises a nucleic acid sequence encoding a human polypeptide.

120. (New) The method of claim 92, wherein said transgene construct is capable of knocking out the expression of a gene endogenous to said first transgenic animal.

121. (New) The method of claim 119, wherein said transgene construct further comprises a promoter wherein the nucleic acid is under the control of said promoter.

122. (New) The method of claim 121, wherein said promoter is a tissue specific promoter.

123. (New) The method of claim 122, wherein said tissue-specific promoter is a promoter preferentially expressed in mammary gland epithelial cells.

124. (New) The method of claim 123, wherein said promoter is selected from the group consisting of a β-casein promoter, a β-lactoglobin promoter, whey acid protein promoter and lactalbumin promoter.

125. (New) The method of claim 121, wherein said promoter is a caprine promoter.

126. (New) The method of claim 119, wherein said nucleic acid encodes a polypeptide selected from the group consisting of a hormone, an immunoglobin, a plasma protein, and an enzyme.

127. (New) The method of claim 119, wherein said nucleic acid encodes a polypeptide selected from the group consisting of an α-1 proteinase inhibitor, an alkaline phosphotase, an angiogenin, an extracellular superoxide dismutase, a fibrogen, a glucocerebrosidase, a glutamate decarboxylase, a human serum albumin, a myelin basis protein, a proinsulin, a soluble CD4, a lactoferrin, a lactoglobulin, a lysozyme, a lactoalbumin, an erythropoietin, a tissue plasminogen activator, a human growth factor, an antithrombin III, an insulin, a prolactin, and an α-1-antitrypsin.

128. (New) The method of claim 92, wherein said second non-human differentiated somatic cell or cell-lines are fibroblasts.

129. (New) The method of claim 128, wherein said fibroblasts are primary fibroblasts.

130. (New) The method of claim 128, wherein said fibroblasts are primary derived fibroblasts.